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DEVELOPMENT OF MICROBIAL AND ENZYMATIC FUEL CELL FOR BIO-INSPIRED POWER SOURCES

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14. ABSTRACT
This report is based on some of the revolutionary concepts described in the Road Map on "Bio-inspired Power Systems" within the AFRL/RW Campus Challenge II. Development of biofuel cell concepts integrated with fuel regeneration and energy storage capabilities are proposed to create a compact and self sustaining power system. The first part of this work identified a sediment based microbial fuel cell that could power small electronics and maintain electrical activity over long periods of time. AFRL/RW does not feel that this work adequately addresses the original scope nor does it provide a basis on which to continue work. The second part of this work focused on the evaluation of the oxidation and photolytic regeneration properties of fuels for enzymatic fuel cells. The top three fuels (glycerol, formaldehyde, and methanol) were electrochemically evaluated in working bioanodes. Despite the high viscosity of glycerol, the energy dense compound can be used in high concentrations making it a better fuel source than the organic alcohols for the enzymatic fuel cells.

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FOR THE DIRECTOR:

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Technical Director	Program Manager
Assessment and Demonstrations Division	

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Development of Microbial and Enzymatic Fuel Cells for Bio-Inspired Power Sources

Research Report Prepared for the

Air Force Research Laboratories Munitions Directorate
(AFRL/RW)

Submitted By

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Program Manager's Note: This work began with a very ambitious scope. The contractor planned to use the expertise of multiple collaborators to address this scope. However, the technical effort collapsed late into the period of performance. AFRL/RW does not feel that the work on microbial fuel cells presented in this report stands as a basis on which to build further work. If you are interested in the technological state of microbial fuel cells or enzymatic fuel cells, please reference Appendix A.

1.0. Background and Need Statement

This report is based on some of the revolutionary concepts described in the Road Map on "Bio-Inspired Power Systems (BIPS)" which was submitted to the AFRL/RW on September 30, 2006 within the Campus Challenge II Problem Solving Competition. The roadmap describes how to apply state-of-the-art nano-engineering and nano-science expertise to adapt lessons-learned from Mother Nature to create revolutionary new approaches to the design and prototyping of high energy density and long-duration power systems. We call these systems Bio-Inspired Power Sources (BIPS) and lay-out a roadmap describing a plan to develop these systems from current levels as low as TRL 1 up to a TRL 4 level within ten years. Development of biofuel cell concepts integrated with fuel regeneration and energy storage capabilities is proposed to create a compact and self sustaining power system with power densities significantly higher than that of the state-of-the-art batteries. The system is envisioned to integrate a biofuel cell with a bio-inspired capacitor (electric eel) and a self-sustaining fuel source.

In this roadmap, we referred to the electric eel or ray as one of the most powerful living species on the earth which can generate, store and release significant amount of electrical energy. The total stored energy in the electric organ discharge (EOD) of electric ray "Torpedo Marmorata" can be up to 38 kWhr (135 MJ) equivalent to a power $>10^5$ W.

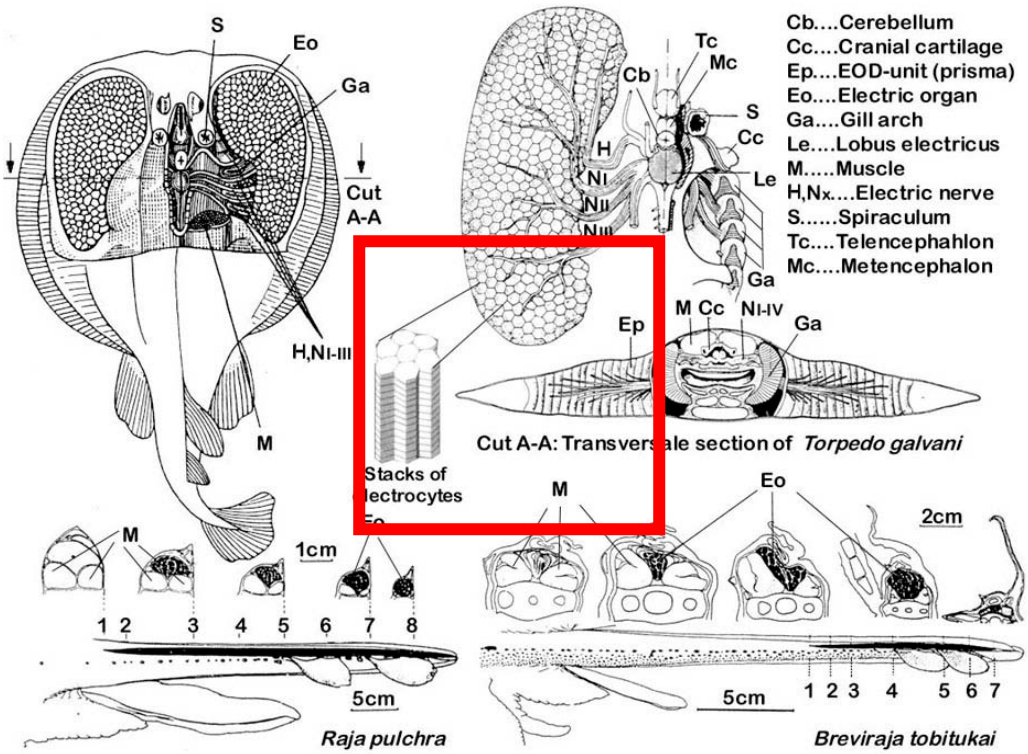
Figure 1 shows the anatomy of electric ray and EOD in which the stacks of electrocytes resemble an electric circuit in series with wired capacitors. These organs are capable of generating strong electric shocks to stun enemies or prey as well as weak electric fields for navigation and signaling which are administered at will.

Research on electric fish opened the path to modern electrophysiology and, through the scientific endeavor of *Alessandro Volta*, led to discovery of the laws of the capacitor and the invention of the electric battery. Alessandro Volta, a professor at the University of Pavia, invented the electric battery in 1799. There is no doubt that the invention of the battery was a landmark. A reflection of the electric organs was to be of importance for Volta's invention of the electric battery (Figure 1). Volta would call it an '*organe eléctrique artificiel*' not only for its similar shape but, also, because, in his opinion, the battery resembled the natural organ in being capable of producing electricity by the 'mere contact of conductive substances'. Volta's interest in the physiological aspects of electrical influence in animal organisms was genuine, and the results he obtained are of great importance, not only because they led to the invention of battery, but also because of their intrinsic biological relevance.[1]

Based on this background information, the objectives of this research effort are to develop microbial and enzymatic fuel cells which can be utilized as autonomous power sources for long operation times. The data presented in this report reveal that biofuel cells are promising candidates

[1] *M. Piccolino et al., Trends in Neurosciences Vol. 25, 51-57 (2002)* 5

for compact power sources which can be further developed through design engineering for unique applications such as pulse power systems.



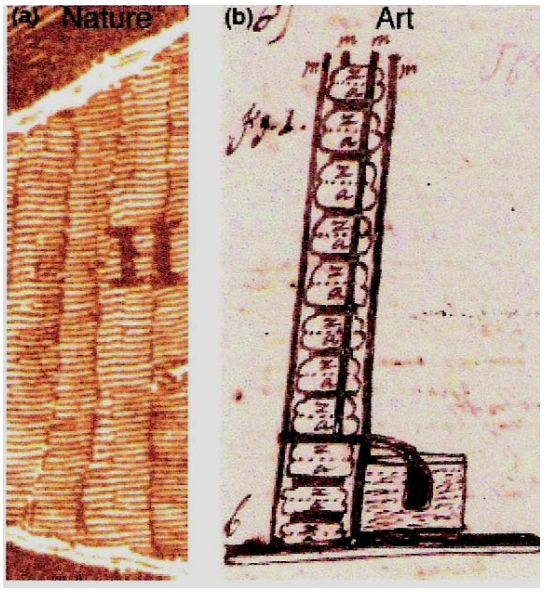


Figure 1: *Anatomy of electric ray and electric organ discharge (EOD); stacks of electrocytes (highlighted in the frame) resemble an electric circuit in series with wired capacitors. These organs are capable of generating strong electric shocks to stun enemies or prey as well as weak electric fields for navigation and signaling which are administered at will. http://www.sbg.ac.at/ipk/avstudio/pierofun/ray/eod.htm*

(a) The structure of the electric organ of fish, with its columns of membranous disks, inspired Alessandro Volta to assemble, in stack-like manner, several disks of two different metals and humid element, thus, leading to the invention of electric battery; (b) drawing from Volta's draft of the communication of battery invention.[1]

2.0. Development of Microbial and Enzymatic Fuel Cells for Bio-Inspired Power Sources

In this reporting period, an overview of the recent developments on "Biofuel Cells" was published and is located in Appendix A and summarized here. In general, fuel cells provide electricity by moving electrons from a fuel to an anode, through an electrolyte, forming a circuit, and expelling them at the cathode in an oxidation reaction. Bioelectrochemical or biological fuel cells (BFCs) follow similar designs of most inorganic fuel cells with the exception that the catalysts employed are not expensive transition metal elements, but cost effective and abundant biological organisms (or their components).[2] Biological fuel cell systems that use intact, living micro-organisms and the metabolic pathways therein as catalysts are categorized as microbial fuel cell or MFCs. The simplest MFCs are sediment based and created by simply immersing an electrode (anode) in anoxic sediment, rich in organic matter, and placing a corresponding cathode into relatively oxygen rich water above the anode, as used in this report.[3] Enzymatic fuel cells (EFCs) differ from MFCs as they use only the relevant biochemicals (enzymes) in engineered systems to directly produce electricity from a variety of substrates. EFCs typically have higher power densities than MFCs, but are frequently plagued with problems associated with incomplete oxidation. Research in the last 10 years has resulted in the development of MFCs with high efficiency (> 80 %) at power densities in the $\mu W/cm^2$ and long lifetimes (> 1 year) and miniature EFCs with higher power densities (> mW/cm^2), but much shorter active lifetimes (typically 7-17 days). Therefore the use of MFCs vs. EFCs will depend on specific applications.

2.1. Microbial Fuel Cells (F. Dogan, Missouri S&T)

Research activities to develop long lasting and high power density microbial biofuel cells were focused on identifying naturally occurring bacterial communities in sediments obtained from various locations such as freshwater (lakes, rivers) and salt water (ocean). Figure 2 shows the location of marine sediments in Gig Harbor near Seattle, WA where the samples were collected. After a low tide, the sediment with ocean water was sampled into a polyethylene bottle and brought to our laboratory at the Missouri University of Science and Technology in July 2006. Since then, electrical power was generated using the same sediment in our lab without the addition of nutrients or any other modifications. The cell which consists of an anode imbedded in the sediment and the cathode immersed into the ocean water is shown schematically in Figure 3. Porous graphite blocks (3 x 1 x 1 inches) were used as electrodes which were connected to the load using insulated copper or titanium wires. While microbial fuel cells using marine sediments showed a relatively high open voltage and power, the performance of biofuel cells containing fresh water sediments from various rivers and lakes were very low. Hence, marine sediments were the preferred source of bacterial communities for monitoring and data collection of biofuel cells in our lab.

---

[2] Bullen, R.A.; Arnot, T.C.; Lakeman, J.B.; Walsh, F.C. *Biosens. Bioelectron*. **2006**, *21(11)*, 2015-2045.

[3] Tender, L.M.; Reimers, C.E.; Stecher, H.A., III; Holmes, D.E.; Bond, D.R.; Lowy, D.A.; Pilobello, K.; Fertig, S.J.; Lovely, D.R. *Nat. Biotechnol.* **2002**, *20(8)*, 821-825.

Characterization of the cells by electrical measurements were conducted using Solartron 1281 Multiplexer with eight channels combined with Solartron 1255B Frequency Response Analyzer (Farnborough, Hampshire, UK). This system can sequence a potentiostat to any of eight different test cells for high performance multichannel testing and automated monitoring. Corr Ware and ZPlot software packages were used for data analysis by combining impedance measurements with conventional DC electrochemical techniques.

Figure 4 shows an increase of the open circuit voltage (OCV) as a function of time for a completely discharged cell obtained under short circuit conditions for an extended period of time. The data from Figure 4 are plotted in Figure 5 using a double logarithmic scale to reveal time dependent reactions while the OCV increases. The initial increase of the open circuit voltage is relatively fast (within six hours $OCV \sim t^{1/3}$) reaching approximately 0.6 V, followed by a slower increase ($OCV \sim t^{1/10}$) so that a maximum OCV of 0.87 V is obtained within a few days. Two fitting lines indicate a two stage process until the OCV reaches the maximum value. Reaction kinetics leading to a two-stage process during the recovery period of the biofuel cell, is currently not well understood and requires further studies.

Another important characteristic of a biofuel cell is the charge storage capacity in the cell, i.e. ability to generate certain current or electrical power for an extended period of time. A galvanostatic method was used to investigate the storage capacity of the biofuel cell. The cell was discharged at a constant current for an extended period of time by monitoring the cell voltage. Corresponding time dependences for different values of discharge current and voltage drop as a function of time are shown in Figure 6.

At a given discharge current, the voltage decreases initially and stabilizes after a certain time indicating that recharging of the biofuel cell and discharging at a constant current and voltage are in equilibrium. This approach allows determining of the current-voltage characteristics of the cell and electrical power generated by the cell at a constant load for an extended period of time. Due to the porous structure of the graphite electrodes, the total cell power was plotted in Figure 7 instead of the power density which may be determined using non-porous electrodes.

I-V characteristics and the power generated using the biofuel cell are shown in Figure 6. The data reveal the open circuit voltage (OCV=0.87 V max) and time necessary to recover 80 % of the OCV after complete discharge of the cell (~6 hours). Electrical power of ~20 μW at a discharge current of ~50 μA is generated for extended period of time. Blinking lights of LEDs assembled in a low voltage flasher circuit (Figure 8) were utilized for a visual demonstration of the biofuel cell.

Another important characteristic of the biofuel cell is that the electrical charge can be stored and released within a short period of time. Hence, the cell can be considered as a self charging electrolytic capacitor. Examples of this charge-discharge behavior are shown in Figure 9. Under various load conditions, the cell voltage first decreases followed by the recovery of the voltage after the load is disconnected (open circuit condition). Figure 10 shows the time dependence of the cell voltage under various loads which is similar to that of electrolytic capacitors. The effective capacitance "C" of the cell can be calculated using the following equation:

$$\mathbf{C=I*t/(U-Uo)}$$

Corresponding results are summarized in Figure 11 for various discharge current values (I) and the time during the discharge process (t) based on the change in voltage (U-Uo) in Figure 10. It is revealed that the effective capacitance remains relatively constant at low discharge currents as a function of time.

Impedance spectra data, shown in Figure 12, reveals high frequency behavior of the cell which has ~12 Ohm electrolyte resistance. This resistance is connected in series with the electrolytic capacitor which results in an increase of Z'' at low frequencies.

Figure 13 reveals the performance of the cell under nearly short circuit conditions. Significantly higher power is generated during a short discharge time in comparison to long discharge times under a constant load of the cell as shown in Figure 7. Maximum power produced under the short circuit conditions of the cell is 4 mW with a discharge current of ~9 mA.

Summary and Outlook

Long term studies on the performance and stability of microbial biofuel cells were conducted using marine sediments and graphite electrodes. While generating electrical power, the fuel cell behaves like an electrolytic capacitor by storing electrical charge. With an open circuit voltage of OC V= 0.87 V, charge loading capacity of the cell was ~20 μW at a discharge current of ~50 μA for an extended period of time. Maximum power produced under short circuit conditions was 4 mW with a discharge current of ~9 mA.

An important characteristic of the cell is that electrical power was generated in our lab for more than 3 years without the addition of nutrients or any other modifications. This indicates that the bacterial community is self sustaining and able to generate electricity as an autonomous power source.

Future studies will include understanding of the nature of energy supplied from the environment to the cell. Higher power densities can be obtained by optimizing the design and electrode configuration of the cell. It is expected that such modifications will result in significantly improved performance of microbial fuel cells as autonomous and long-lasting power sources.

Another area of research interest is to develop miniaturized biofuel cells which are connected in series. This stack configuration would mimic the electric organ discharge (EOD) of the electric ray as shown in Figure 1. It is envisioned that miniaturized microbial fuel cells can serve as self sustaining energy systems for pulsed power applications in analogy to the electric fish.

Figure 2: Location of marine sediments collected from Gig Harbor near Seattle, WA

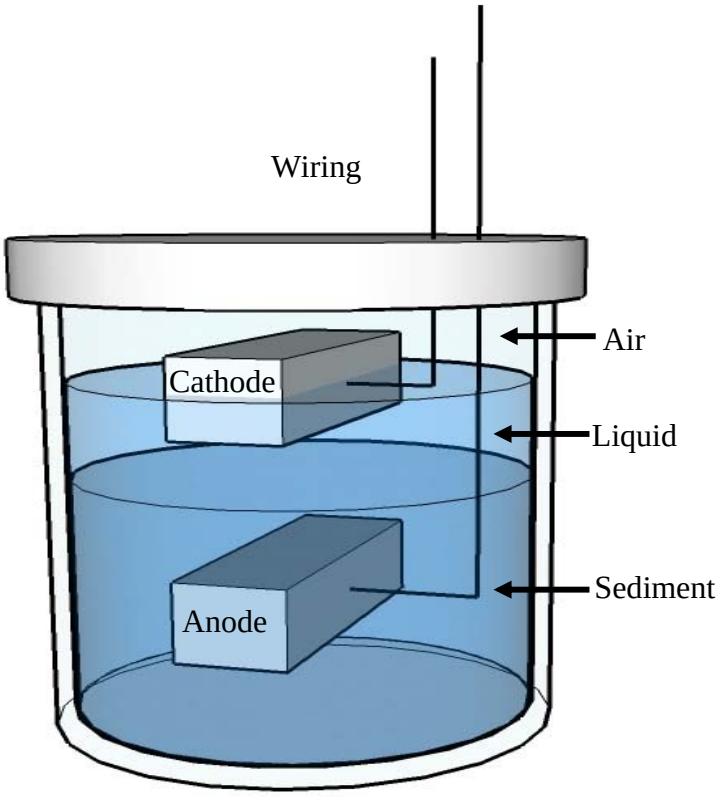


Figure 3: Schematic of the biofuel cell assembly showing the electrodes configuration in marine sediment.

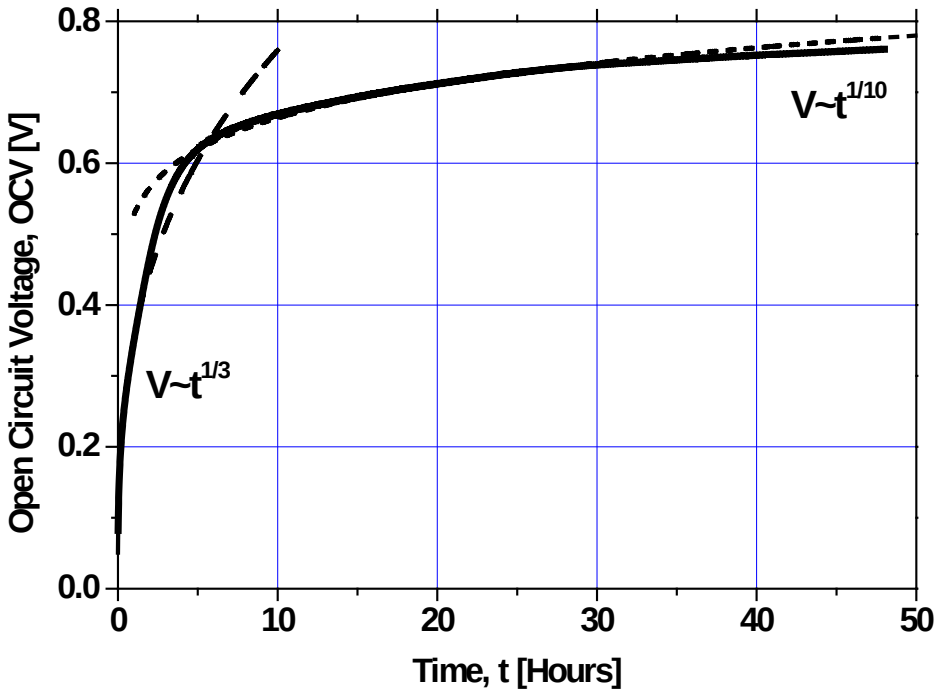


Figure 4: Recovery of open circuit voltage as a function of time after fully discharging the cell.

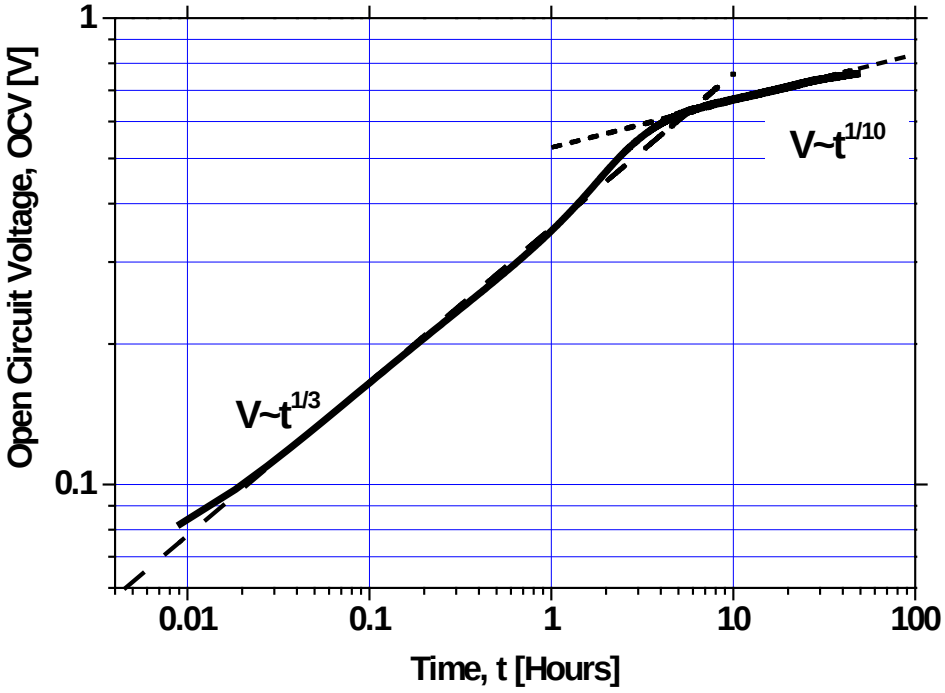


Figure 5: Recovery of open circuit voltage as a function of time after discharging the cell (double logarithmic scale).

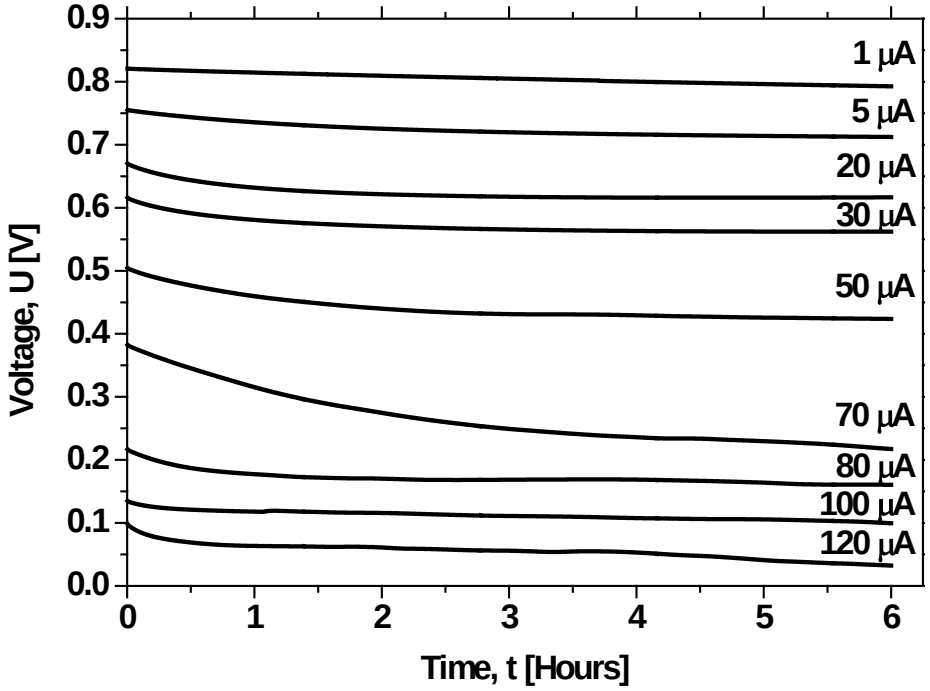


Figure 6: Time dependence of the cell voltage measured at different values of discharge current.

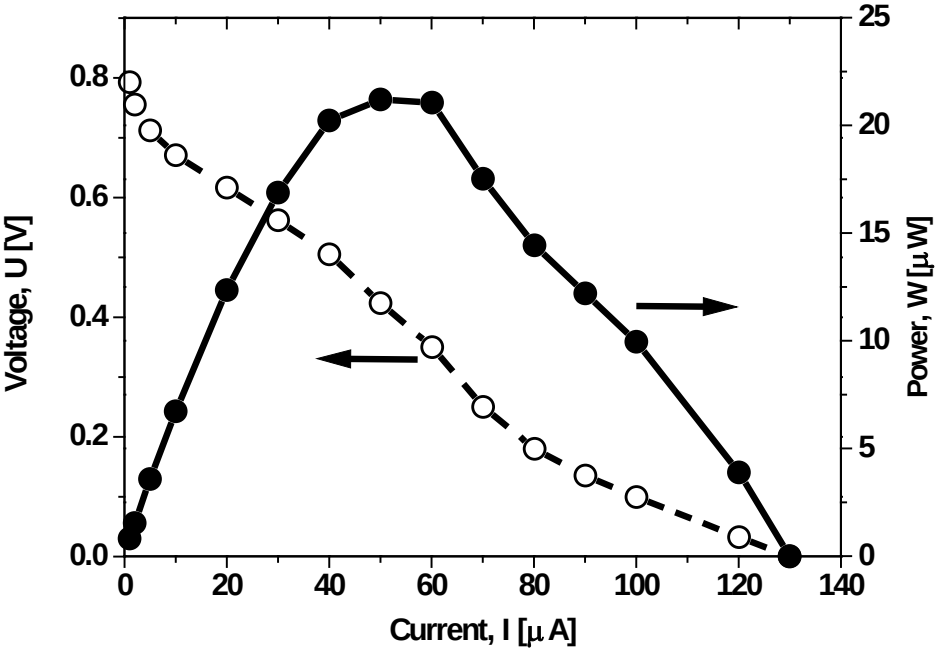


Figure 7: Current to voltage characteristics (dashed line) and power produced by the cell (solid line) at a constant load for extended period of time.

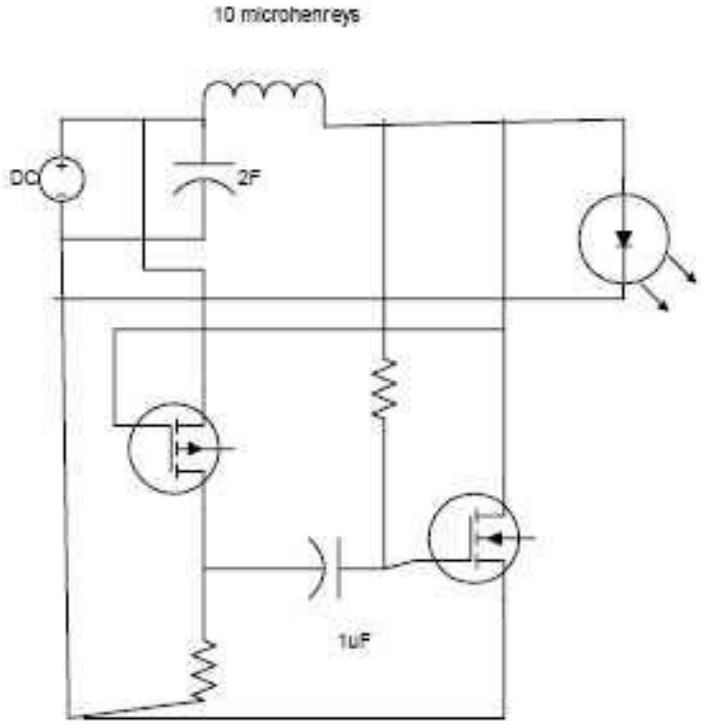


Figure 8: Simplified drawing of low voltage flasher circuit used to power LEDs in visual demonstration.

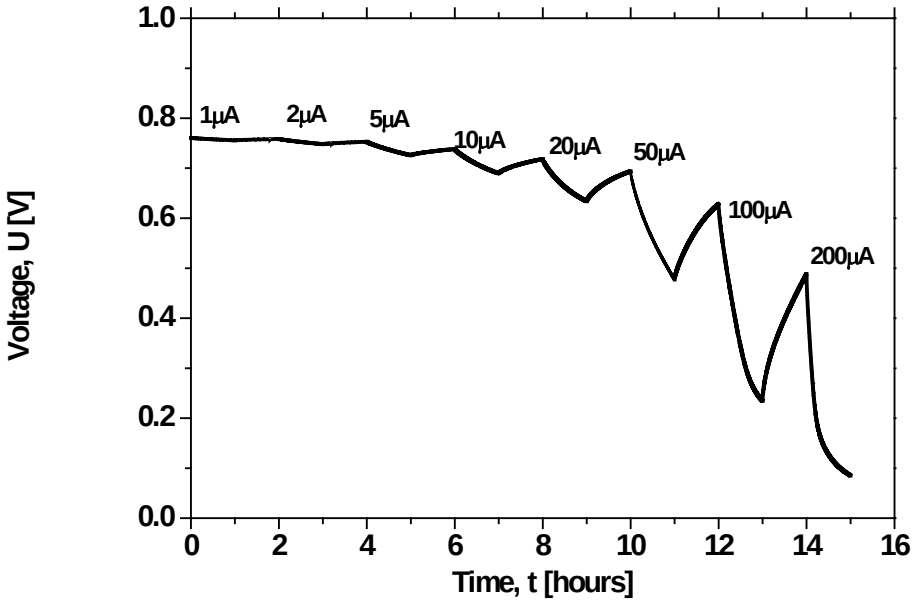


Figure 9: Time dependence of the cell voltage measured at different values of discharge current combined with the voltage recovery process (one hour intervals) at open circuit conditions.

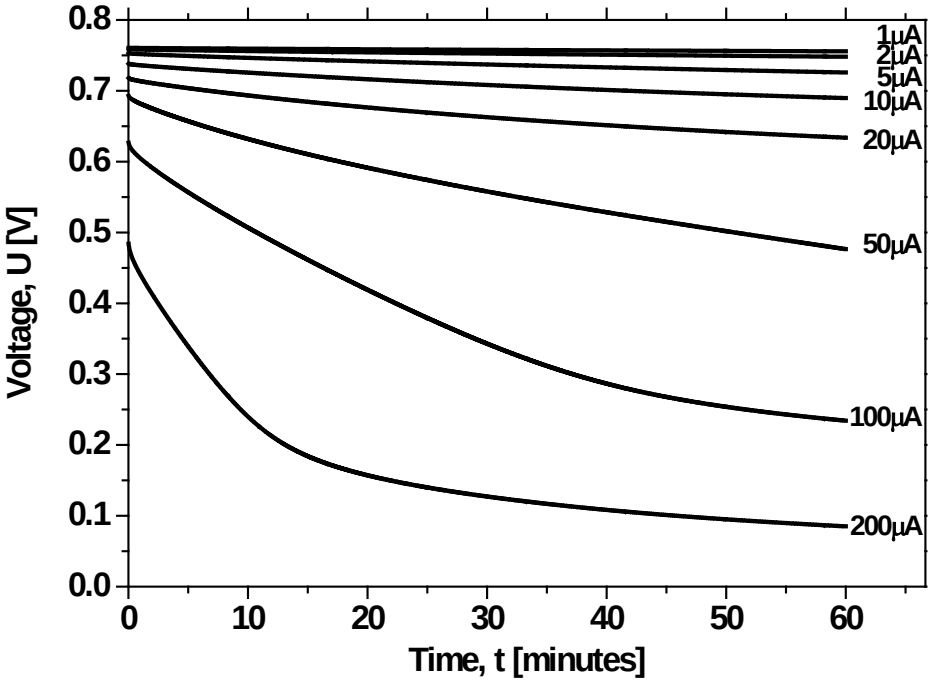


Figure 10: Time dependence of the cell voltage measured at different values of discharge current after charging of the cell for one hour.

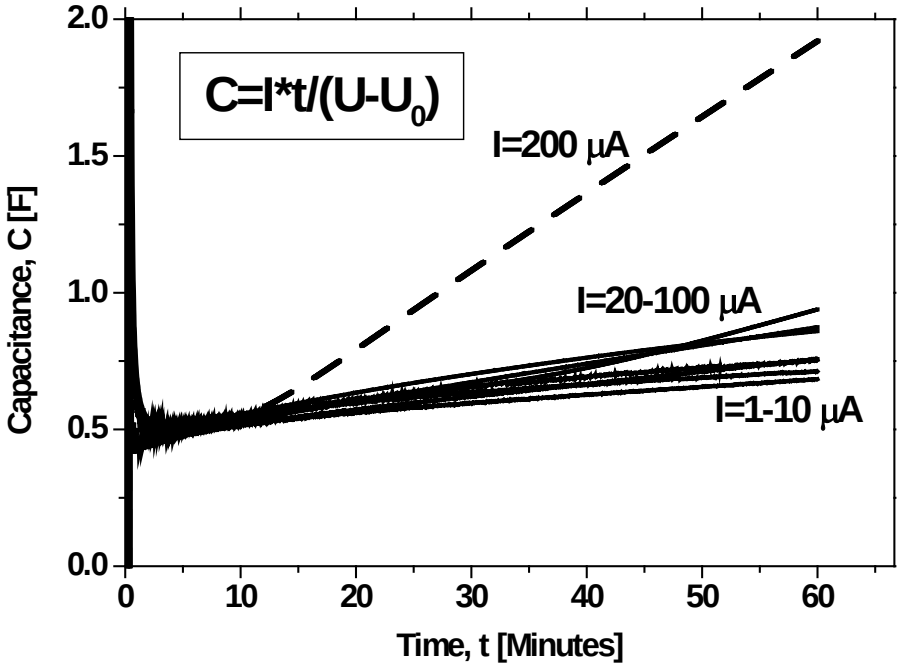


Figure 11: Time dependence of effective capacitance of the cell measured at different values of discharge current.

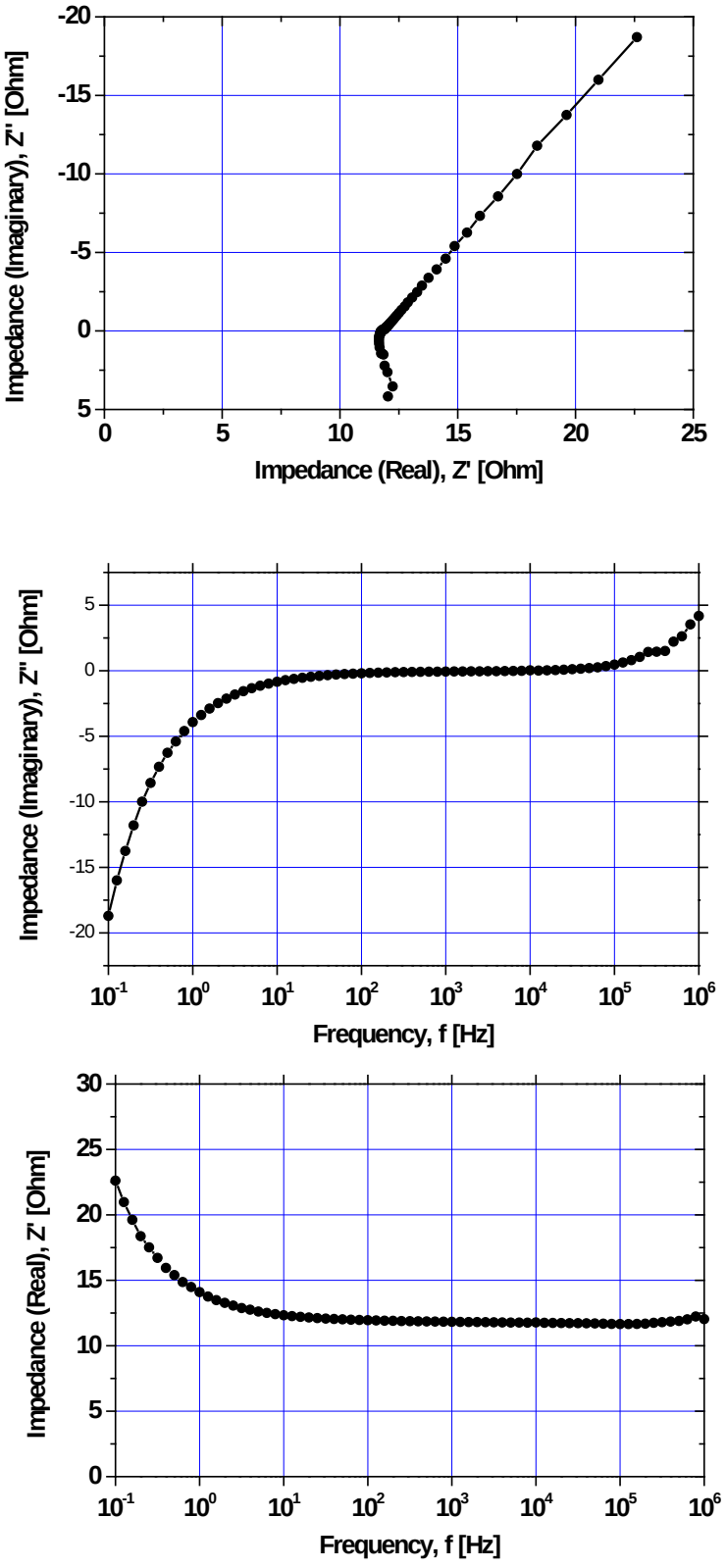


Figure 12. Impedance spectra of the biofuel cell shown as (Z'' –Z', Z'' – f, and Z' –f).

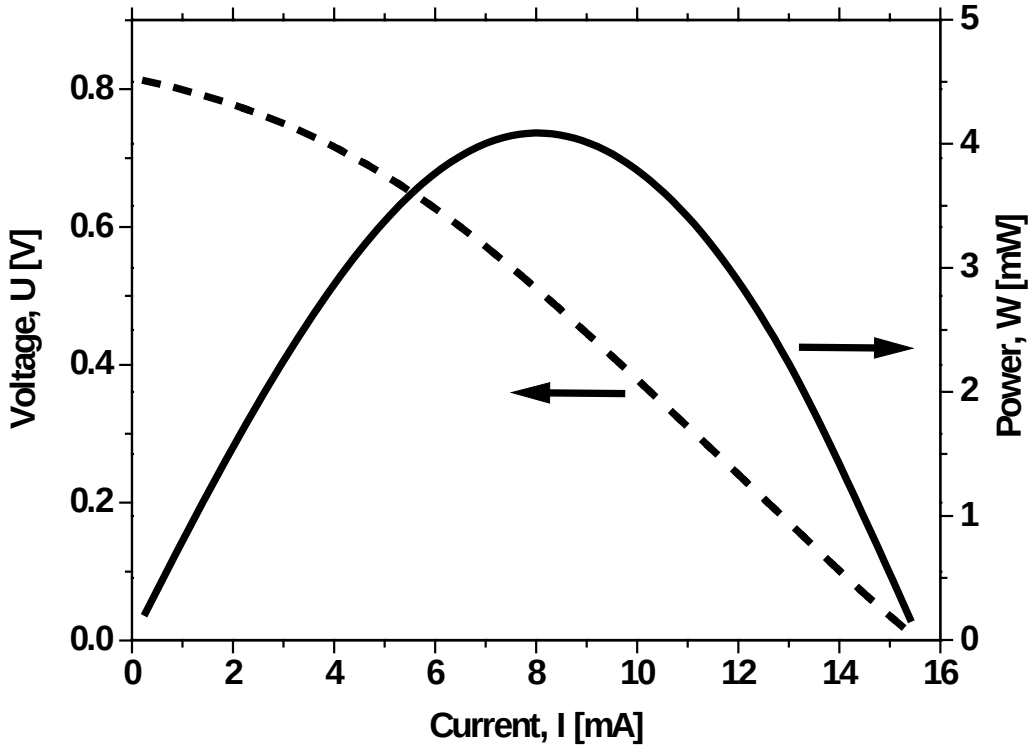


Figure 13. Current-voltage characteristics (dashed line) and electrical power (solid line) measured at a high discharge rate of the cell.

2.2. Enzymatic Fuel Cell (S. Minteer, SLU)

Biofuel cells are normally divided into two categories: microbial biofuel cells and enzymatic biofuel cells. Microbial biofuel cells employ living cells to catalyze the oxidation of fuels at the anode surface. They have the advantage of being able to catalyze complete oxidation of biofuels and have long lifetimes (up to 3-5 years), but are plagued by low power densities (0.0010 -0.09 mW/cm^2) due to slow transport of fuel across cellular membranes. Enzymatic biofuel cells employ enzymes to catalyze the oxidation of fuels at the anode surface. They have the advantage of higher power density (1.65 –4.1 mW/cm^2), but are plagued by incomplete oxidation of fuel and frequently low lifetimes (8 hours to 10 days). Saint Louis University has made advances in enzymatic fuel cell lifetimes over the last 8 years, so enzymatic biofuel cells can have lifetimes of greater than1 year due to the development of a novel enzyme immobilization membrane that three-dimensionally constrains the enzyme while providing a buffered pH and a hydrophobic environment that mimics the cellular environment. The enzymatic fuel cell part of this project was focused on the development of a high energy density fuel for enzymatic biofuel cells with photolytic fuel regeneration.

Subtask 1: Fuel identification and downselection

Candidate fuels were computationally evaluated for their suitability both for oxidation by the bioanode and for their photolytic regeneration by the Photolytically Driven Electro-Chemical (PDEC) system. The energy density of fuels undergoing partial oxidation and ability to regenerate the fuel from a partial oxidation product was evaluated when identifying the optimal fuel. A computation model was generated to determine the optimal fuel based on the energy density of a single step oxidation of the fuel by a dehydrogenase enzyme, sufficient activity of the dehydrogenase enzyme (>40 U/mg to ensure sufficient current densities), the stability of the fuel and oxidized product (which is critically important to the ability to photolytically regenerate the fuel), and the ability to undergo direct electron transfer. The following fuels were considered: ethanol, methanol, glycerol, butanediol, propanol, xylulose, sorbitol, mannitol, formaldehyde, formic acid, acetaldehyde, glyceraldehyde, glucose, lactate, pyruvate, glycerate, hydroxybutyrate, malate, arabinose, galactose, propanediol, lactaldehyde, octanal, aminopropanol, tartrate, xylose, ribose, aldose, sorbose, fructose, mannose, cyclohexanol, thiamine, cellobiose, benzaldehyde, and succinate. The top 3 most ideal fuels were chosen from the model and they are (in order): glycerol, formaldehyde, and methanol. Table 1 is the complete list of candidate fuels with their overall energy density (for complete oxidation of the fuel in a traditional biofuel cell format) and their energy density for single enzyme oxidation (partial oxidation to a product that can be used by the PDEC photolytic regeneration system to regenerate the fuel). Formic acid was not chosen as a candidate, because its product is a gas (carbon dioxide) that is difficult to use to regenerate the fuel with the PDEC system. Glyceraldehyde and glycerate were also not chosen as ideal fuels due to the more optimal enzyme and chemical properties for glycerol.

Table 1: Overall energy density and energy density for the photolytic regeneration-based biofuel cell system.

Fuel	Energy Density (Whr/L)	Single Enzyme (Whr/L)
acetaldehyde	5506	1420
aldose	6304	1282
arabinose	6870	838
benzaldehyde	9617	779
butanediol	7798	890
cellobiose	8447	408
cyclohexanol	9943	763
ethanol	5440	1360
formaldehyde	4398	2155
formic acid	1875	2105
fructose	6720	679
galactose	6660	679
glucose	6720	679
glyceraldehyde	6304	2565
glycerate	4565	2332
glycerol	6260	3261
hydroxybutyrate	6183	859
lactose	8447	408
lactaldehyde	5482	1084
lactate	4325	1066
malate	4426	953
mannitol	7056	662
mannose	6720	679
methanol	4040	1962
octanal	8927	508
propanediol	6820	1081
propanol	7501	1062
pyruvate	4594	1127
ribose	6571	798
sorbitol	7038	649
sorbose	6950	710
succinate	5471	1049
tartrate	3743	931

Subtask 2: Development of Enzymatic Bioanodes for Glycerol, Formaldehyde, and Methanol

Bioanodes were formed for each of the three most ideal fuels: glycerol, formaldehyde, and methanol. These bioanodes were optimized for high open circuit potential and high current

density. Open circuit potential was optimized by improving the method of attachment for direct electron transfer systems. Current density was optimized by maximizing enzyme loading and increasing mass transport through the enzyme immobilization membrane.

The glycerol bioanodes were formed employing pyrolloquinoline quinine (PQQ)-dependent alcohol dehydrogenase. They were tested in a biofuel cell configuration with an air-breathing commercial platinum electrode as the cathode. Figure 14 is a representative power curve for the glycerol biofuel cell. This biofuel cell provides relatively low power density due to the high viscosity of the glycerol fuel.

The methanol and formaldehyde biofuel cells were more complicated. The PQQ-dependent enzymes that can do direct electron transfer did not result in fuel cells that could actively catalyze the oxidation of methanol or formaldehyde, so mediated fuel cell systems were investigated. It was found with a poly(methylene green) mediated nicotinamide adenine dicnucleotide (NAD)-dependent aldehyde dehydrogenase anode that although the bioanode had activity to acetaldehyde, it did not have activity toward formaldehyde, so significant power could not be generated from the formaldehyde bioanode as shown in Figure 15. However, a poly(methylene green) mediated NAD-dependent alcohol dehydrogenase anode resulted in a high performance methanol biofuel cell as is shown in Figure 16. The power density is low for these systems due to slow mass transport of NAD/NADH in the membrane instead of slow transport of the fuel.

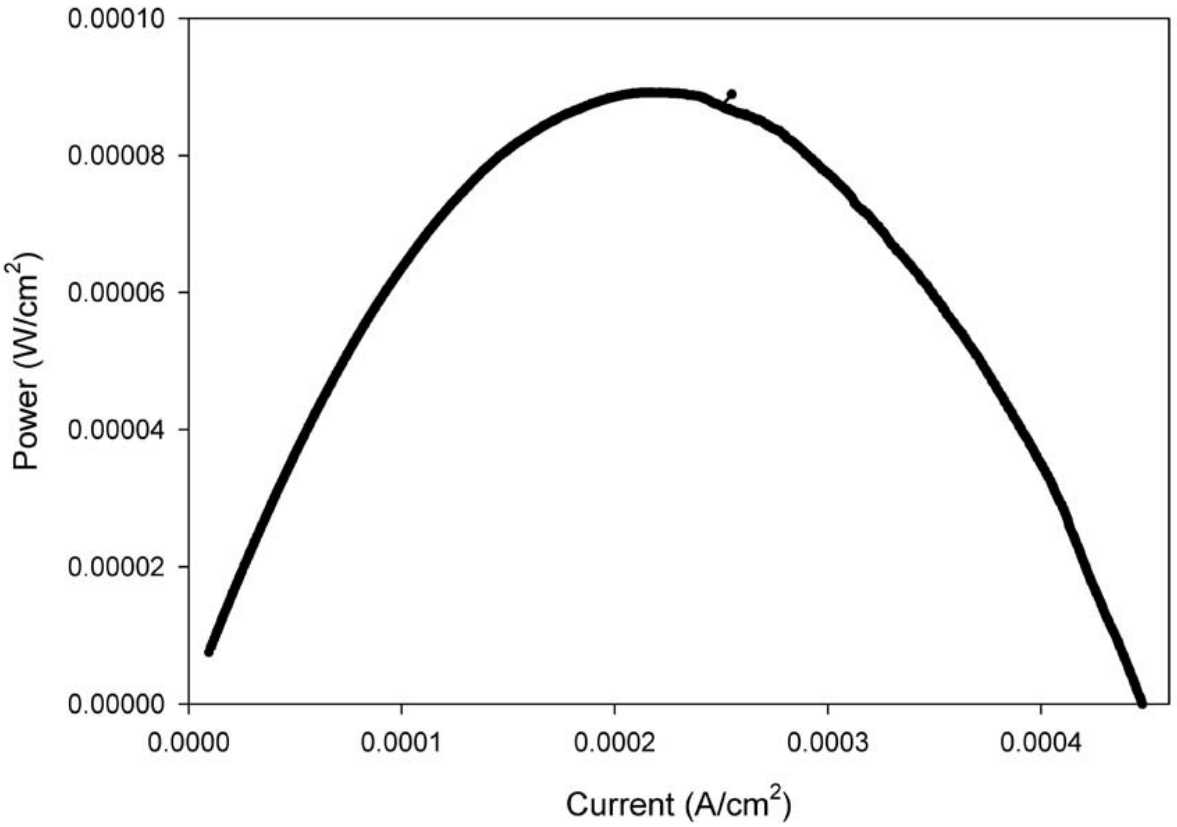


Figure 14: Representative power curve for glycerol biofuel cell fabricated directly on Toray carbon fiber paper employing direct electron transfer.

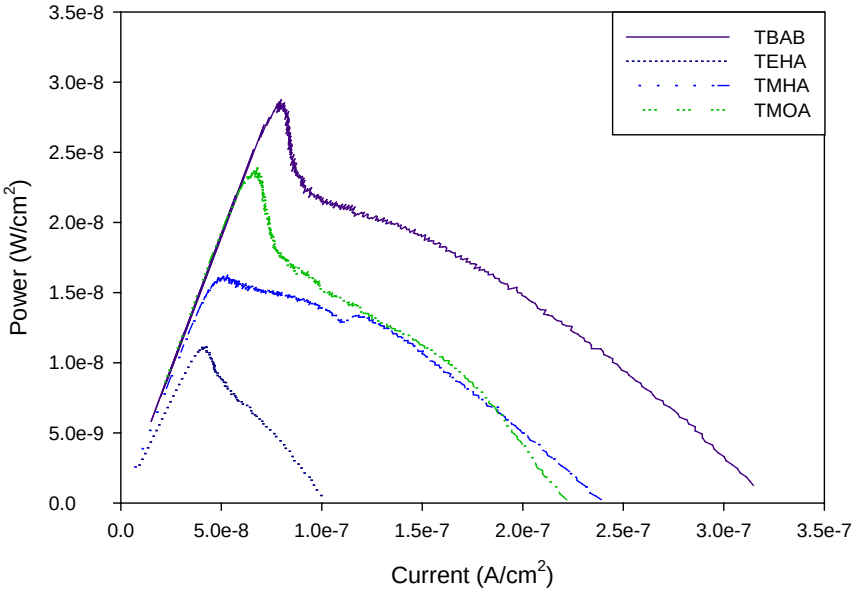


Figure 15: Representative power curves for formaldehyde biofuel cell employing aldehyde dehydrogenase. Several enzyme immobilization membranes were tested (tetrabutylammonium bromide modified Nafion (TBAB), triethylhexylammonium bromide modified Nafion (TEHA), trimethylhexylammonium bromide modified Nafion (TMHA), and trimethyloctylammonium bromide modified Nafion (TMOA)), but no immobilization membrane resulted in significant formaldehyde activity (power of greater than a microwatt per square centimeter).

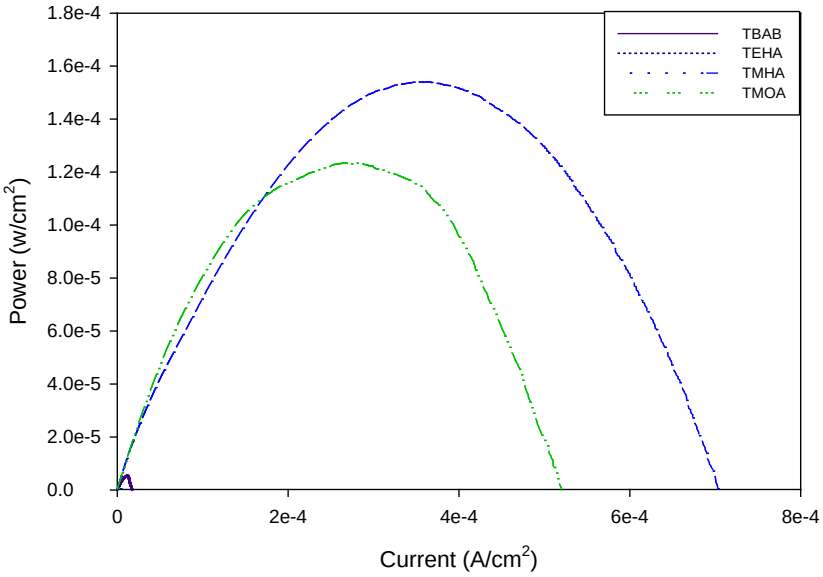


Figure 16: Representative power curves for NAD-dependent methanol biofuel cells mediated with poly(methylene green). Dehydrogenase immobilization in TMHA modified Nafion and TMOA modified Nafion resulted in biofuel cells with significant power densities compared to dehydrogenase enzyme immobilized in TBAB modified Nafion or TEHA modified Nafion.

Summary and Outlook

In this study we computationally evaluated energy dense fuels that could reasonably be deployed in enzymatic fuel cells to power devices such as micro aerial vehicles (MAVs). The three most promising fuels (glycerol, formaldehyde, and methanol) were electrochemically evaluated in working bioanodes. Although these fuels have great energy potential, the practical application of each came with a unique set of problems such as fuel transport (glycerol) and mass transport through the membrane (formaldehyde and methanol). Although methanol appears to produce the highest power density of all the fuel cells, it is actually less suitable than glycerol. High fuel concentration is required to maintain a high power density fuel cell that can continuously power an energy hungry device like the MAV. Therefore, despite the viscosity issues associated with glycerol, it can be packed into a bioanode in a much higher concentration than the organic alcohols. Future work should include genetically enhancing the activity of the glycerol enzymes to improve oxidation of the fuel and provide more power density. In addition, improvements to the electrode structure should be examined to improve the transport of the glycerol.

APPENDIX A

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Biofuel Cells

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Abstract
Living cells oxidize a wide variety of fuels by employing enzymes as catalyst. It has long been recognized that microbes can generate voltage and deliver current. Researchers have been studying the harvest of stored energy from fuels to electrical energy via a biofuel cell. A biofuel cell is a fuel cell that converts the chemical energy stored in a fuel into electrical energy through the catalytic activity of living cells or their enzymes. Biofuel cells have the advantage of not requiring precious metals and being able to generate fuel from a wide variety of possible fuel sources (including ethanol, sugars, proteins, fatty acids, and more). However, the main problems that have hindered the advancement of biofuel cell power supplies are low power densities for microbial systems and short lifetimes of the enzyme catalyst. Continued research, however, has enabled research to overcome these limitations leading to novel applications of biofuel cells as bioinspired power sources.

INTRODUCTION

Fuel cells are viewed as environmentally compatible and efficient energy conversion systems. A fuel cell works much like a battery with external fuel supplies. Chemical fuels are electrochemically converted into electricity at high efficiencies without producing significant amount of pollutants such as nitrogen oxides as compared with combustion engines. Various types of fuel cells are commercially available such as proton exchange membrane, molten carbonate, solid oxide fuel cells (SOFCs), etc., and are capable of generating power in the kW and MW range. Although the power outputs reported for biofuel cells thus far are usually small, in mW range, this is expected to change in the future. Biofuel cells have a number of unique potential uses such as power sources for sensors and microactuators or pacemakers in the body. Future applications of biofuel cells encompass, e.g., novel power sources for autonomous micro air vehicles (MAV), which are inspired by biological power generation inherent to, e.g., insects and birds. The following sections give a brief overview on biofuel cells categorized by the type of biocatalyst employed: microbial and enzymatic fuel cells.

BIOLOGICAL FUEL CELLS

In general, fuel cells provide electricity by moving electrons from a fuel to an anode, through an electrolyte, forming a circuit, and expelling them at the cathode in an oxidation reaction. Bioelectrochemical or biological fuel cells (BFCs) follow similar designs of most inorganic fuel cells with the exception that the catalysts employed are not expensive transition metal elements, but cost effective and abundant biological organisms (or their components).[1] The focus of BFC research has been on the synergistic combinations of the metabolic pathways of organisms and the engineering of systems to efficiently harvest the energy output. Many different types of BFCs exist as they are broadly defined as any electron-generating oxidation or reduction that rely on biocatalysts for part of its activity.[2] Such an overarching definition has been subsequently divided into a host of other subcategories as seen in Fig. 1. F1 F1

Microbial Fuel Cells

Biological fuel cell systems that use intact, living microorganisms and the metabolic pathways therein as catalysts are categorized as microbial fuel cells or MFCs. The most current and concise definition of an MFC involves electrons moving from substrates (inorganic or organic) via the metabolic pathways of intact, living microbes resulting in a current when these electrons are accepted by an anode and are sustained by the supply of substrate.[3,4] Enzymatic fuel cells (EFC) differ from MFCs as they use only the relevant biochemicals

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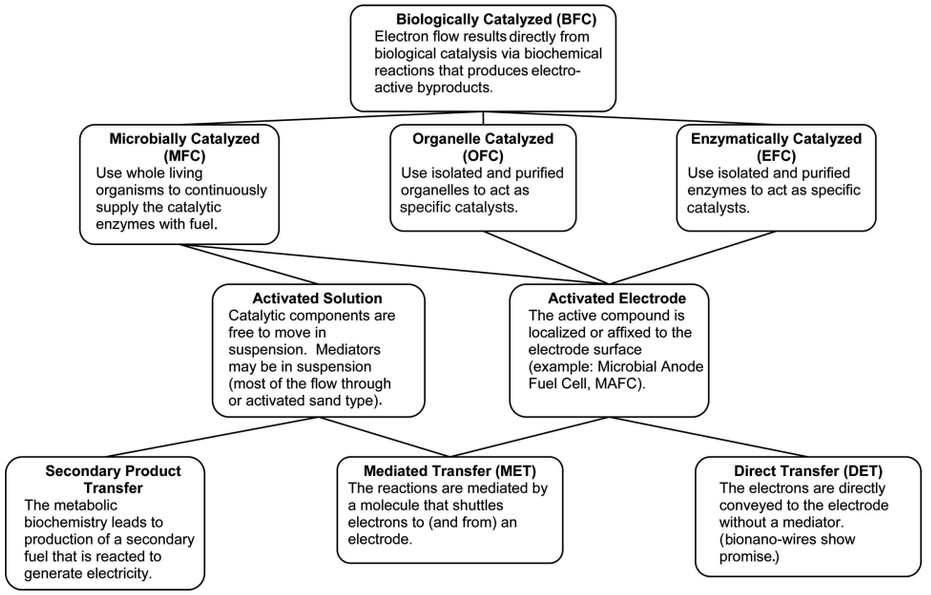

Fig. 1 Classification diagram of biological fuel cells. *Source*: Modified from Biofuel cells and their development, by Bullen et al. In Biosens. Bioelectron. **2006**, *21*, 2015–2045.[1]

(enzymes) in engineered systems to directly produce electricity from a variety of substrates. These specific fuel cells are treated in more detail in another section of this entry.

Previous literature had also classified systems that employ the microbial bioconversion of organic substrates into *products* that are then used to generate electrical energy in conventional fuel cells.[5] For example, a system that uses hydrogen gas produced from microorganisms, either photosynthetically or fermentatively, and then delivered to polymer electrolyte membrane (PEM) fuel cell or a SOFC has previously been considered an MFC.[6] However, a recent paper delineated distinctions between the types of MFCs[7] with the biodigestion type technology being re-classified as microbial-based biofuel cell (MBBC).[8] Another noteworthy variation of the MBBC results in hydrogen production. The bioelectrochemically assisted microbial reactor (BEAMER) uses bacterial fermentation and a small amount of external power to generate hydrogen at the cathode of a MFC.[9]

Thus far, MFC systems in the literature have been mostly chemosynthetic in nature; however, photosynthetic systems do exist. These direct photosynthetic microbial fuel cells (DPMFCs) differ from similar MBBC systems in that the electrical current is produced directly, instead of from the products of photosynthetic microorganisms.[10]

Before further classification of MFCs can be made, it is important to note two main components of MFC systems: mediators and membranes. As with any conventional fuel cell, electrons must be shunted into a circuit, while the balancing cation must be transported along a different path via an external circuit to create a high potential via the redox potential of the chemical reactants at the anode and cathode. Microbial fuel cells may or may not use an artificial electron shuttle or mediated electron transfer (MET) to move electrons from the bacteria to the anode. These electron transport mediators help increase the efficiencies of the fuel cells as the microbial membrane itself isolates the reduced products generated inside of the organism. Therefore, a chemical shuttle is employed to accept electrons from the organism and transport them to the anode. MFCs that use mediated designs fall into one of the three categories: (1) a free mediator that moves electrons between the electrode and either attached or suspended microbes; (2) a mediator that is bound to the membrane of microbial cells to provide a conduit from the interior of the cell to the electrode; (3) a mediator that is bound to the electrode itself. A diagram of these three systems can be seen in Fig. 2. F2 F2
Much research has thus far looked into MET designs because direct electron transport designs (DET) have traditionally resulted in low efficiencies.[11] However, MFCs that employ DET are preferred as they avoid the toxicity issues associated with chemical mediators. Certain bacteria like *Shewanella putrefaciens* and many members of the family *Geobacteraceace* have shown DET. These particular strains also have been found to possess structures such as nanowires that possibly allow transmission of electrons directly from organism-to-organism or from organism to a solid electron acceptor such as an anode.[12] Overall, the

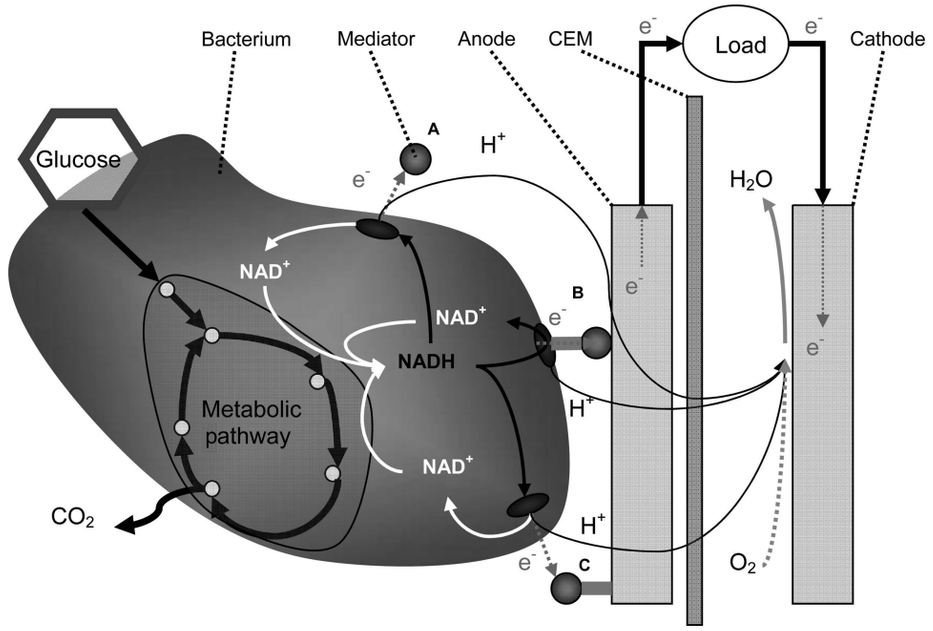

Fig. 2 Representation of the basic operation principles of a microbial fuel cell. Fuel, in the form of organic substrates (glucose in this example) is metabolized by the bacteria. Electrons liberated during metabolism are shuttled to terminal electron acceptors in the membrane of the bacteria (brown ovals) and can be conveyed to the anode via mobile redox shuttles (A), mediators absorbed to the membrane or nanowires (B), or through mediators bound to the anode surfaces (C). Electrons flow through the circuit from the anode to the cathode through the load. Protons are also produced in excess and these are allowed to pass through the cation exchange membrane (CEM) to the cathode chamber. The final electron acceptor (oxygen in this example) reacts with the protons and electrons. *Source*: Modified from Microbial fuel cells: novel biotechnology for energy generation, by Rabaey, K. & Vestraete, W. In Trends Biotechnol. **2005**, *23* (6), 291–296.[19]

bacteria best suited for mediator-less MFCs are those that can couple the oxidation of organic matter to metal reduction. The most common MFC, in anaerobic systems, utilize Fe(III) as the preferred oxidizer because of its similar reduction potential to oxygen (Fe(III) = 0.76 V to O_2 = 0.82 V). Therefore, the iron-reducing bacteria (IRB) process is more thermodynamically favorable than other anaerobic respiratory pathways.[13,14]

Mainly owing to considerations about kinetics and the reduction of overvoltages, MFCs employ some method of separation between the electrons and the electron acceptors used at the cathode to avoid possible formation of undesired electron sinks in the anode compartment. Both modes of electron transfer, DET or MET, can be created with or without the use of a formal membrane, but they both require some sort of separation to avoid short-circuiting the cell. The simplest MFCs family is sediment type MFCs and they do not rely on a formal membrane. This type of MFCs is created by simply immersing an electrode (anode) in anoxic sediment, rich in organic matter, and placing a corresponding cathode into relatively oxygen rich water above the anode.[15] In the case of sediment cells, the ''membrane'' is a suitably thick layer of sediment that maintains an oxygen gradient between the anode and cathode. Other materials have included clay,[16] nanoporous polymer filters,[17] as well as proton exchange membranes like Nafion™ (Dupont; http://www.dupont.com) or Ultrex™ (Membranes International; http://www.membranesinternational.com).

In general, MFC devices may or may not contain a membrane; are mediator or are mediator-less; or undergo some combination or both components are excluded. Most MFC models were developed to mimic the processes that were observed in natural systems. Findings by microbiologists indicate that bacteria could utilize soluble components in the environment such as humic acids or extracellular cytochromes to transfer electrons extracellularly.[18] Electron mediators may be quite benign such as the humic acids or quite toxic like 2,6-dichlorophenol-indophenol (DCPIP). Further, the added complexity of membranes and additional mediators to the MFC has not proven to be any more electrochemically cost efficient.[19]

Microbial fuel cells convert reduced substrates to usable energy utilizing a vast array of enzymatic processes within the cell. However, the processes simply rely on an electronegative gradient that is maintained by cellular compartmentalization, to create a resulting ''flow'' of energy. For the sake of simplicity, bacterial

metabolism can be summed up mathematically as ideal cell voltages (ΔE) or the electron motive force (emf) that are only theoretically constrained by the potentials of the oxidizer and the fuel compounds ($E^0_{ox} - E^0_{fuel}$). To clarify, the electron donor is defined as the fuel component or energy source.[20] Ultimately, the electrons donated from the fuel are accepted by the oxidizer and the active potential available for work is determined by the following, assuming normal operating conditions of a MFC (298 K, 1 M Glucose, pO_2 of 0.2 atm and pH of 7).[3]

$$\begin{aligned} \Delta E_{emf} &= E^0_{ox} - E^0_{fuel} \\ &= (0.820\,V) - (-0.43\,V) \\ &= 1.25\,V \end{aligned} \quad (1)$$

Realistically, there are irreversible losses as a result of kinetic limitations of electron transfer, electrolyte resistance, and unfavorable concentration gradients. Thus, the highest reported open circuit voltage (OCV) is 0.80 V with a theoretical OCV of between 1.25 V and 1.14 V.[21] The losses are grouped into overpotentials and ohmic resistance that contribute to the total overvoltage of the system. These relationships are better explained by the following equations from the literature:[1]

$$E_{emf} - E_{cell} = \text{Overvoltage} \quad (2)$$

$$E_{emf} - E_{cell} = (\Sigma\eta_{anode} + |\Sigma\eta_{cathode}| + IR_\Omega) \quad (3)$$

$\Sigma\eta$ is the sum of the overpotentials for the anode and cathode, respectively, and are categorized as current dependent losses attributed to activation losses, bacterial metabolic losses, and mass transport losses. IR_Ω is the ohmic resistance of the system that encompasses both the resistance to flow of electrons through electrodes and interconnections and the resistance to the flow of ions through the electrolytes and membranes.[3] For the MFC, the activation potential has the greatest negative impact on the system. Basically, the amount of energy released in the metabolism/catabolism cycles of bacteria is proportional to the difference in reduction potential between the electron donor and electron acceptor with the greatest losses attributed to the electrochemical properties of the materials involved in the MFC.[19]

As for applications of MFCs, the simplest have produced enough power for successful trials on unattended sensors in marine environments[15] and have also been considered for large scale power production in coastal areas.[22] Another primary branch of MFC application research is the harvest of usable energy from the treatment of municipal waste water.[23–25] A spin-off of water treatment MFC research is the use of MFCs as the basis of an effective water quality sensor.[26–28] Finally, there is significant interest in MFCs for powering autonomous vehicles and robots.[29]

As of yet, the MFCs cannot compete with the relatively high power density of inorganic fuel cells. To take the MFC from biochemical curiosity to keystone energy generation technology, comprehension of the basic process is necessary and much more research will focus on finding synergistic combinations of biology and engineering to increase the output.

Enzymatic Fuel Cells

Enzymatic fuel cells employ a particular type of protein called oxidoreductase enzymes as the electrocatalyst at the anode and/or the cathode of a fuel cell. In early research, the enzymes were placed in the anolyte or catholyte solution,[30] while today, most researchers immobilize the oxidoreductase enzymes at the electrode surfaces by covalent binding, sandwich techniques, or entrapment.[31] Covalent binding of the enzyme to the electrode surface (typically, by diimide chemistry) is a good technique to ensure even coverage of the enzyme on electrode surfaces and to ensure that the enzyme is in close contact with the electrode surface; however, covalent binding typically decreases the catalytic activity and stability of the enzyme. Therefore, there is no literature example of high activity (i.e., high power density) or long lifetime biofuel cells employing covalently bound enzymes. Sandwich techniques involve physical trapping/placement of the enzyme between the electrode and a polymer layer (i.e., laying a polymer coating on top of the enzyme to hold it at the electrode surface). Sandwich techniques are useful for ensuring that the enzyme is near the electrode surface, but typically have stability and heterogeneity problems. Entrapment is a popular technique that employs trapping the enzyme within a polymeric matrix, so that it will prevent the enzyme from diffusing out and may stabilize the enzyme by both a three-dimensional encapsulation and an optimal chemical microenvironment for optimal catalytic activity. Polymeric matrices can be tailored via hydrophobic and hydrophilic groups to increase activity and stability, but frequently leaching is a problem along with transport problems associated with decreased mass transport rates and increased diffusion distances to the electrode surface.

Enzymatic fuel cells typically employ oxidases or dehydrogenases at the anode to partially oxidize the fuel. These oxidases and dehydrogenases require coenzymes (or cofactors) to function and maintain activity. The coenzymes that are typically employed with bioanodes of biofuel cells are nicotinamide adenine dinucleotide (NAD^+), flavin adenine dinucleotide (FAD),

and pyrolloquinoline quinone (PQQ). FAD and PQQ are bound coenzymes, as the coenzyme is bound to the enzyme and does not need to be added to the anolyte solution or immobilized within the enzyme immobilization membrane. Direct electron transfer (DET) is the ability of the enzyme to communicate directly with the electrode surface without the need for any additional redox species. Both FAD and PQQ types of enzymes are theoretically capable of direct electron transfer. For instance, PQQ-dependent alcohol dehydrogenase can oxidize ethanol and transfer the electrons directly from the enzyme to carbon electrode surfaces,[32] and glucose oxidase (GOX), which is a FAD-dependent enzyme, can oxidize glucose and transfer the electrons directly to carbon electrodes.[33] Enzymatic fuel cells typically employ oxidases,[34] laccases,[35] or peroxidases[36] at the cathode to reduce oxygen or peroxide. These enzymes typically contain multiple metal centers, which allow them to undergo direct electron transfer at many electrode surfaces.

Although many enzymes are capable of undergoing DET, the majority of enzymatic biofuel cells in the literature are based on mediated electron transfer MET. This is because the metal, FAD, or PQQ moieties are typically buried deep in the protein shell and it is difficult to orientate the enzyme to the electrode in such a way to minimize the distance from the metal, FAD, or PQQ moieties to the electrode, while still providing sufficient space for the substrate to diffuse to the active site. Fig. 3 compares MET to direct electron transfer. Mediated electron transfer is a mechanism for electron transfer where the enzyme transfers an electron to/from another redox species (redox mediator), and then the redox mediator is the species that actually transfers the electron to/from the electrode. Most enzymatic biofuel cells employ mediators, because typically the rate of DET is less than the rate of MET, i.e., higher current densities can be achieved with MET bioanodes and biocathodes, and DET is extremely difficult to achieve experimentally. The problem with mediators is threefold: (1) instability of the mediator, (2) transport limitations of the mediator between the enzyme and the electrode surface, and (3) voltage losses associated with the potential of the mediator being less than the potential of the redox reaction of the enzyme. Owing to these three principal problems as well as attempts to alleviate these three problems, much research has been done on finding the best mediator and the best method for mediation.[30,37] Researchers have explored both organic and inorganic mediators to find a mediator with good stability that has a redox potential that is as close to that of the oxidation of NADH as possible.[30] A variety of organic mediators have been studied for the anode, including phenazines,[38] dyes,[39,40] and quinone.[41] Although organic mediators have been employed in solution, the future of the technology is in finding appropriate ways to immobilize the mediators at the electrode surface while optimizing transport and stability. At the cathode, osmium-based mediators have been attached to polymeric structures to form redox polymers.[34,35] This has been a successful approach to optimize stability, transport, and voltage losses.

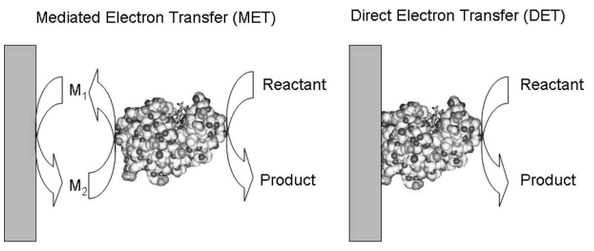

Fig. 3 Comparison of mediated and direct electron transfer. M_1 and M_2 denote the redox mediator in its two different oxidation states.

Enzymatic biofuel cells typically have higher power densities than microbial biofuel cells, but are frequently plagued with problems associated with incomplete oxidation. Therefore, the applications of enzymatic biofuel cells are different from those microbial biofuel cells. Enzymatic biofuel cells have in vivo, implantable applications, where partial oxidation of fuels, such as glucose in the blood stream, produce metabolites that can still be used by the organism. Further, its nearly unlimited supply of fuel in the plant or animal makes the fuel cell energy density less of an issue. Also, since enzymatic biofuel cells have higher power densities than microbial biofuel cell, they have applications where battery size is a major concern (e.g., portable electronics, sensors, etc.).

CONCLUSIONS

Over the last decade, there has been a great deal of research on both MFCs and EFCs. The results of those studies are the development of MFCs with high efficiency (>80%) at power densities in the $\mu W/cm^2$ and long lifetimes (>1 year) and miniature EFCs with higher power densities ($>mW/cm^2$), but much shorter active lifetimes (typically 7–14 days). Therefore, the use of microbial versus EFCs will depend on specific application. In applications where there is limited fuel available, a need to utilize a wide variety of fuels or a need to utilize an impure fuel, or a need to ensure there are no oxidation byproducts left in the waste stream, MFCs are the obvious choice. For applications where a higher current drain, small size, or catalytic selectivity is needed, EFCs are the preferred choice. However, there are applications where a hybrid MFC/EFC is

a better choice than either the MFC or the EFC, i.e., power generation from sediment or ocean sand for remote sensing. In membrane-less systems (sediment type MFC), the theoretically optimum fuel cell would be a hybrid MFC/EFC employing a microbial bioanode to completely oxidize the wide variety of fuels in sediment (causing high efficiency and fuel diversity) with an enzymatic biocathode to improve selectivity and activity, resulting in both higher open circuit potentials and higher current densities.

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